

Diapause Response of the Boll Weevil (Coleoptera: Curculionidae) to Feeding Period Duration and Cotton Square Size

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Abstract

Distribution of the boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), in the United States has been greatly reduced by eradication efforts. Still, it remains a key pest of cotton (*Gossypium* spp., [Malvales: Malvaceae]) in the New World, and has proven difficult to eliminate from Mexico and from southern Texas. In those regions, improved knowledge of boll weevil overwintering ecology may benefit efforts by eradication and management programs. Adult diapause in the boll weevil is well documented, but influences of the feeding period duration between adult eclosion and assessment of diapause remain unstudied. We examined diapause incidence and associated survival for weevils fed for 7, 14, or 21 d after adult eclosion. Diapause incidence of females was less influenced by feeding duration compared with males. For males, highest diapause incidence occurred after 14 d of feeding compared with 7 or 21 d. Host-free survival tended to be higher after 14 d of feeding compared with 7 or 21 d, although many weevils were long-lived (≥ 80 d) after each feeding period duration. Males exhibited higher survival compared with females, and survival was higher for weevils fed large flower buds (squares) compared with smaller squares. Survival was most influenced by temperature; longevity increased with decreasing temperature except at the lowest temperature (12.8°C). These results suggest an optimal feeding period for induction of diapause and maximized host-free longevity. These findings may permit improved timing of late-season insecticide treatments aimed at reducing overwintering populations, and thereby improve effectiveness of eradication and management programs.

Key words: flower-bud size, dormancy, temperature, cotton, host-free survival

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), is one of the most important pests of cotton (*Gossypium* spp. [Malvales: Malvaceae]) in the New World, and is especially important in South America (Scataglini et al. 2006, de Oliveira et al. 2016). Within the United States, progress by eradication programs has reduced the economically important range of the boll weevil to extreme southern Texas. However, populations in South Texas and Mexico are proving intractable, and pose a persistent threat to re-infest eradicated areas. Available control tactics, whether in an eradication or management context, are generally limited to insecticide applications made to the cotton crop, and cultural methods focused on maximizing weevil mortality either during the cropping season, or during the noncotton fallow season (Brazzel

et al. 1996, Leser et al. 1996). Especially in this latter case, designing and implementing control strategies requires an astute understanding of the overwintering ecology of the boll weevil, and of associated implications for weevil survival in the absence of the cotton host.

The boll weevil is widely recognized to overwinter in a state of dormancy, characterized as an adult diapause by Brazzel and Newsom (1959). Since that report, numerous articles have reported on various aspects of the diapause. However, most reports have focused on the various environmental cues inducing diapause such as photoperiod, temperature, diet, or some combination of these factors (Earle and Newsom 1964, Lloyd et al. 1967, Cobb and Bass 1968, Mangum et al. 1968, Tingle and Lloyd 1969, Sterling 1972, Carter and Phillips 1973, Wagner and Villavaso 1999). Most

investigators appear to have accepted photoperiod as an important cue inducing diapause in the boll weevil, and physiological studies of diapause have often featured experimental weevils induced into diapause by photoperiod, either alone or in combination with another factor (Lambremont et al. 1964, Nettles and Betz 1965, Betz and Lambremont 1967, Nettles et al. 1972, Mitlin and Wiygul 1976, Taub-Montemayor et al. 1997a, b). Other reports have questioned either the primary role of photoperiod in diapause induction (Carter and Phillips 1973, Keeley et al. 1977) or the larger set of putative determinants of diapause including photoperiod (Rummel and Summy 1997). Although the literature regarding boll weevil diapause induction is voluminous, reports of this phenomenon are rife with inconsistencies. Spurgeon et al. (2003) and Spurgeon and Raulston (2006) discussed likely sources of these marked inconsistencies, including lack of adequate experimental designs and replication, failure to account for physiological age in studies incorporating temperature treatments, lack of detailed control over or descriptions of experimental diets, and inconsistent application of the morphological criteria indicating diapause.

Spurgeon and Raulston (2006) reported results in which boll weevils held and fed as a group, as opposed to weevils fed singly, elicited the diapause response in a reproducible fashion. Responses observed for weevils from the subtropics were similar to responses of weevils from the southern temperate zone. Their study was unable to demonstrate any effect of photoperiod. In addition, the effect of temperature was limited to influencing the temporal pattern over which the diapause characters were exhibited. However, the age at which diapause was assessed influenced the perceived diapause response. Recent results (Spurgeon et al. 2018) expanded on those diet studies and found that under a putative diapause-suppressing photoperiod (13:11 [L:D] h), diapause response increased with increasing development of the food items (squares [flower buds] vs bolls [fruit], smaller squares or bolls vs larger squares or bolls). These results also indicated the estimated diapause response was influenced by weevil adult age (the duration between eclosion and diapause assessment) in a fashion similar to that reported by Spurgeon and Raulston (2006). Serial dissections at either three (Spurgeon et al. 2018) or four ages (Spurgeon and Raulston 2006) were used in these studies because of uncertainty regarding the appropriate age for diapause assessment. Spurgeon and Suh (2017) obtained high levels of diapause at different temperatures by feeding groups of weevils a boll diet, also under a putative diapause-suppressing photoperiod (13:11 [L:D] h). They found the influence of adult age on the estimated incidence of diapause varied with temperature, although females tended to exhibit the diapause characters at earlier ages compared with males.

Earlier reports reflect a variety of adult ages at diapause assessment. Although numerous studies assessed diapause at 2 wk of age, the temperatures among or within those studies varied from 21 to 32°C (Lambremont 1961, Earle and Newsom 1964, Betz and Lambremont 1967, Harris et al. 1969). Other investigators used feeding periods as short as 7 or 10 d before diapause assessment (Mangum et al. 1968, McCoy et al. 1968, Carter and Phillips 1974, Mitlin and Wiygul 1976) or as long as 20–35 d under a variety of constant and fluctuating temperature regimes (McCoy et al. 1968, Tingle and Lloyd 1969, Jenkins et al. 1972, Cole and Adkisson 1983). Wagner and Villavaso (1999) and Wagner et al. (1999) examined the diapause response under a range of simulated environments but assessed diapause at weevil ages ranging from 4 to 43 d and from 13 to 37 d, respectively. Spurgeon (2008) observed that host-free survival tended to be higher when adult weevils were fed 21 d compared with adults fed 28 d. These results suggested a survival penalty for the extended feeding period. Therefore, it appears that both

estimation of the diapause response and associated host-free survival may be influenced by the duration of the adult feeding period. Our objective was to examine the influence of feeding period duration on the incidence of diapause and patterns of subsequent host-free longevity, and the potential modifying influence of diet (square size) during the feeding period, and temperature during the host-free survival period, on the survival effects of feeding period duration.

Materials and Methods

We conducted two separate experiments to assess the influence of adult feeding period duration on incidence of diapause, and on subsequent host-free survival. Adult boll weevils of known age were reared from larvae in infested squares which were collected from cotton plants in the field. Weevils for the first experiment were collected near Weslaco, TX, and weevils for the second experiment were collected near College Station, TX. Infested squares were held within environmental chambers at $29.4 \pm 2^\circ\text{C}$ with a 13:11 (L:D) h photoperiod until periodic examinations indicated the presence of pupae. These rearing conditions were selected for consistency with earlier reports (Spurgeon and Raulston 2006; Spurgeon and Suh 2017, 2018). In addition, day length during crop maturation and harvest, when diapause would be induced, is longer than 12 h in the southern portions of the boll weevil range in Texas. Once pupae were present they were harvested daily, placed in groups of 30–50 on a layer of moistened vermiculite within 100- × 15-mm Petri plates, and returned to the environmental chambers. Plates of pupae were examined twice daily for newly eclosed adults, which were separated by sex using the tergal notch method (Sappington and Spurgeon 2000). Single-sex groups of 40 weevils were confined within 473-ml cardboard cartons fitted with screened lids. Each carton was supplied water in a 29.5-ml plastic cup that was closed with a plastic cap penetrated by a cotton wick. Cartons of weevils were the experimental units that were assigned to feeding duration, diet, and survival treatments. All squares fed to weevils during both studies were greenhouse-grown, undamaged, rinsed with reverse osmosis water, and had the bracteoles removed before they were provided to the weevils. In all experiments, adult weevils in cartons were held at $29.4 \pm 1^\circ\text{C}$ with a 13:11 (L:D) h photoperiod during the feeding periods before dissection or assignment to a survival cage.

Diapause status was assessed by dissection under water in parafin-lined plates as described by Spurgeon et al. (2003). Criteria distinguishing diapause were presence of a mature, hypertrophied fat body, combined with small testes (long axis length < ½ the combined length of the meso- and metathorax and abdomen) opaque with fat for males, and lack of vitellogenic oocytes, eggs, or follicular relics for females, as recommended by Spurgeon et al. (2003). Weevils with any other combination of fat body or gonadal development were classed as non-diapausing.

Survival cohorts in both studies were held within 20- × 20- × 20-cm screened acrylic cages. Each cage was provisioned with a snap-cap vial containing water with a cotton wick extending through the vial lid. A sheet of pleated and crumpled brown craft paper (30 × 40 cm) was included in each cage as a refuge for the weevils.

Experiment 1: Feeding Period Duration

Experimental treatments were durations of the feeding period (in days from adult eclosion). Three cartons (replications) of boll weevils of each sex were assigned to each of three feeding period durations (7, 14, or 21 d). The 40 weevils in each carton were provided eight

squares (6–9 mm diameter), which were replaced daily. The numbers of squares provided were adjusted to approximate the desired ratio of one square per five weevils when more than two weevils died in an individual carton. At the end of each feeding period, 20 weevils from each carton were randomly assigned to a survival cohort. Each of these weevils was marked on the right elytron using a non-toxic paint pen (Speedball Painters, Hunt Manufacturing, Statesville, NC) and numbered consecutively using a technical pen (Pigma Micron 005, Sakura Color Products, Osaka, Japan). Weevils not selected for survival cohorts ($30 \leq n \leq 63$ per sex by feeding period combination) were immediately dissected to determine diapause status. These weevils represented a sample size (number of experimental units) of $n = 3$ for each treatment combination of feeding duration, weevil sex, and experiment repetition.

Weevils from a pair of cartons (one of each sex) representing the same feeding duration were combined within a single survival cage. Therefore, a mixed-sex group consisting of one 20-male cohort and one 20-female cohort was assigned to each cage. Cages were held within an environmental chamber maintained at $29.4 \pm 1^\circ\text{C}$ with a photoperiod of 13:11 (L:D) h. Each survival cohort was examined for mortality daily for the first week, on days 9, 11, and 14, and weekly thereafter until all weevils were dead.

Experiment 2: Feeding Period Duration, Square Size, and Survival Temperature

Experimental treatments included the same feeding durations (7, 14, or 21 d) as in the first experiment, which were combined with the effects of size class of squares (small, 5–7 mm diameter; large, 7–9 mm diameter) that were fed to the weevils for the examination of the incidence of diapause. Both of these effects (feeding duration, square size) were combined with temperature (12.8, 18.3, 23.9, or 29.4°C , $\pm 1^\circ\text{C}$) in the subsequent examination of host-free survival. These temperatures were selected for consistency with those used by Spurgeon and Suh (2018). Each combination of square size and feeding duration was represented by four cartons (replications) of weevils of each sex (a total of 48 cartons of 40 weevils each). Single-sex groups of weevils within the cartons were fed at the same rates (one square per five weevils) and frequency as in the first experiment. At the end of each feeding period, 20 weevils randomly selected from each carton were assigned to a survival temperature. The weevils were painted, numbered, and held as mixed-sex groups exactly as in the first experiment, except that survival was assessed weekly until mortality was complete. Weevils not selected for survival estimates ($50 \leq n \leq 73$ per sex by feeding period by square size combination) were immediately dissected to determine diapause status. These weevils represented a sample size of $n = 4$ experimental units for each combination of feeding duration, weevil sex, square size class, and experiment repetition.

Statistical Analyses

All analyses were conducted using SAS software (SAS Institute 2012). The probability of diapause was estimated in the first experiment using a generalized linear mixed model (PROC GLIMMIX) with a binomial distribution, Laplace estimation, and events/trials syntax. The model contained fixed effects of boll weevil sex, feeding duration, and their interaction, and the random effect of replication. A non-negligible interaction between sex and feeding period was addressed by examining the simple effects of each of these factors while controlling for the other effect (Stroup 2013). Where multiple comparisons were made, type I experiment-wise error was controlled using the ADJUST=SIMULATE option, and estimated probabilities

on the data scale were obtained using the ILINK option. Also, survival analyses were conducted using the LIFETEST procedure. Comparisons of the survival functions corresponding to weevil sex were made controlling for feeding period duration and replication using the STRATA statement (Allison 1995). Comparisons among feeding durations were similarly made controlling for weevil sex and replication, and type I experiment-wise error was controlled using the ADJUST=SIMULATE option. Both survival analyses were based on the log-rank statistic.

Incidence of diapause in the second study used the same analytical approach as in the first study except fixed effects of the model were feeding period duration, square size, weevil sex, and all possible interactions. Survival analyses also used PROC LIFETEST to examine respective effects of square size, feeding period duration, weevil sex, and survival period temperature. In each analysis, one of these variables was the independent variable, whereas the others were included as stratification effects.

Results

Experiment 1: Feeding Period Duration

Analyses of diapause incidence did not indicate an influence of feeding period duration (Table 1). The sex main effect and the sex by feeding period interaction were not significant, but both were suggestive of non-negligible effects. Examination of the simple effects of the sex by feeding period duration interaction indicated no differences in diapause response among feeding durations for females, but a significant difference among feeding durations was detected for males (Table 1). Comparisons of the probability of diapause among feeding periods for males indicated a lower incidence of diapause after 7 d of feeding compared with 14 d of feeding ($t = -2.79$, $df = 10$, adjusted- $P = 0.044$; Fig. 1). Also, simple effect tests of sex within feeding period duration indicated a higher probability of diapause for males compared with females after feeding for 14 d (Table 1; Fig. 1). Differences between boll weevil sexes were not detected within other feeding durations (Table 1).

Comparisons among survival functions controlling for weevil sex and experimental repetition failed to indicate differences among the feeding durations. However, the log-rank statistic was suggestive of a difference (log-rank $\chi^2 = 5.94$, $df = 2$, $P = 0.051$; Fig. 2a). Mean (\pm SE) survival time was numerically higher for weevils fed for 14 d (53.9 ± 3.05 d) compared with those fed for 7 (45.5 ± 3.08 d) or 21 d (41.7 ± 2.70 d). Although weevils that were fed 21 d were 7 d older compared with the weevils fed for 14 d when their respective

Table 1. Main effect and simple effect tests (of the weevil sex by feeding duration interaction) of the probability of diapause for adult boll weevils fed for different durations (7, 14, or 21 d) at $29.4 \pm 1^\circ\text{C}$ with a photoperiod of 13:11 (L:D) h

Main or simple effect	F	df	P
Sex	4.86	1, 10	0.052
Duration ^a	1.90	2, 10	0.200
Sex by duration	3.42	2, 10	0.074
Duration within female	0.48	2, 10	0.635
Duration within male	4.13	2, 10	0.049
Sex within 7-d duration	0.16	1, 10	0.698
Sex within 14-d duration	8.89	1, 10	0.014
Sex within 21-d duration	0.65	1, 10	0.438

The analysis used a conditional model with a binomial distribution.

^aDiets were one square (6–9 mm diameter) per five weevils daily.

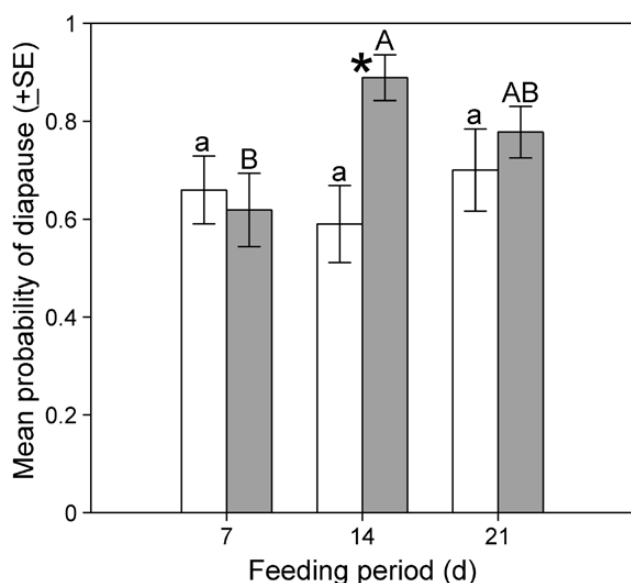


Fig. 1. Mean probability (\pm SE) of diapause for boll weevil adults assessed by dissection after feeding in groups on cotton squares (6–9 mm diameter) for 7, 14, or 21 d at 29.4°C with a photoperiod of 13:11 (L:D) h; females (white bars), males (shaded bars). White bars with a different lower case letter, shaded bars with a different upper case letter, or paired bars marked by an asterisk (*) are significantly different at experiment-wise $\alpha = 0.05$.

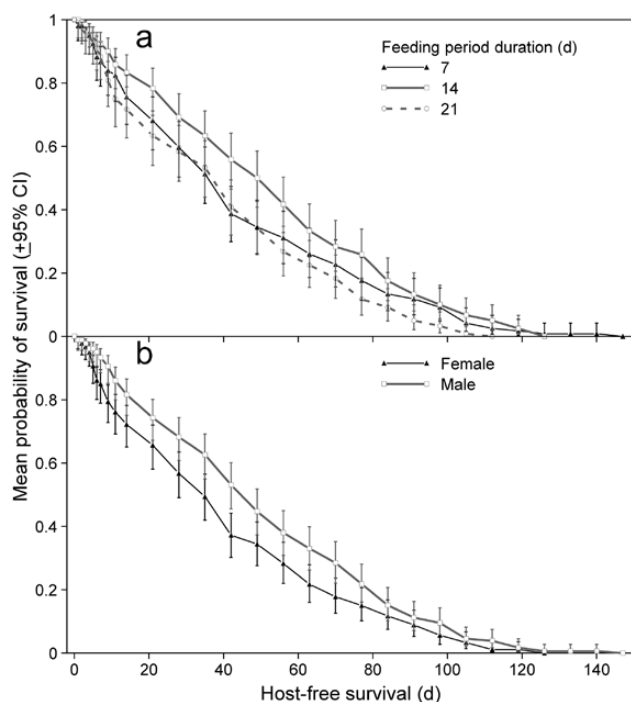


Fig. 2. Mean host-free survival (\pm 95% CI) of adult boll weevils fed in groups on cotton squares (6–9 mm diameter) at 29.4°C with a photoperiod of 13:11 (L:D) h, contrasted by feeding period duration (7, 14, or 21 d) (a), and by weevil sex (b).

starvation periods began, the mean survival time for weevils fed for 21 d was nearly 2 wk shorter than for weevils fed 14 d. Analyses that controlled for feeding duration and repetition indicated longer survival for male weevils compared with females (log-rank $\chi^2 = 7.22$, df = 1, $P = 0.007$; Fig. 2b).

Experiment 2: Feeding Period Duration, Square Size, and Survival Temperature

Analyses of the probability of diapause suggested negligible interactions between weevil sex, square size, and feeding duration, and between square size and feeding duration (Table 2). However, both the sex by square size and the sex by feeding duration interactions were significant (Table 2). Therefore, interpretations of the main effects (sex, square size, feeding period) were not straightforward. Simple effect tests indicated differences among feeding period durations for both male and female weevils (Table 2). Multiple comparisons among feeding durations indicated the incidence of diapause exhibited by female weevils fed for 7 d was higher compared with females fed for 21 d (adjusted- $P = 0.034$). Differences in the probability of diapause between 7- and 14-d (adjusted- $P > 0.999$) and between 14- and 21-d feeding periods (adjusted- $P = 0.068$) were not indicated after adjustments for multiplicity (Fig. 3). In contrast, the probability of diapause in males was higher after 14 d of feeding compared with either the 7-d (adjusted- $P < 0.001$) or 21-d feeding durations (adjusted- $P = 0.001$; Fig. 3). In addition, the incidence of diapause in males was higher after 21 d of feeding compared with the 7-d feeding period (adjusted- $P = 0.002$). Tests of simple effects of weevil sex within feeding durations indicated a higher probability of diapause for females compared with males fed for 7 d, but a higher incidence of diapause for males than for females fed for 14 d (Table 2; Fig. 3). The occurrence of diapause for males and females fed for 21 d was similar (Table 2).

Simple effect tests of the weevil sex by square size interaction indicated the larger squares elicited a more pronounced diapause response by both males and females compared with the smaller squares (Table 2; Fig. 4). In addition, the incidence of diapause for females fed large squares was higher compared with males, whereas both sexes responded similarly to the small squares (Table 2; Fig. 4).

Comparisons of survival functions controlling for weevil sex, square size, and survival temperature indicated significant differences among feeding period durations (Table 3). Multiple comparisons indicated higher survival rates for weevils fed 14 d compared with those fed for 7 or 21 d (Table 3; Fig. 5a). Survival functions of weevils fed for 7 or 21 d were not distinguishable (Table 3; Fig. 5a). Comparisons of male and female survival functions, controlling for square size, feeding period, and survival temperature, yielded results similar to those from the first study. Overall, survival of males was greater than survival of females (Table 3; Fig. 5b).

Comparisons of survival functions corresponding to square size classes, controlling for weevil sex, duration of feeding, and survival temperature, indicated lower survival for weevils fed small squares compared with large squares (Table 3; Fig. 6a). However, the most marked differences among survival functions corresponded to survival temperatures (Table 3; Fig. 6b). Pairwise comparisons among the survival functions indicated differences among all temperature treatments (Table 3). Although most comparisons indicated host-free longevity increased with decreasing temperature, longevity at 18.3°C was greater compared with 12.8°C (Table 3; Fig. 6b).

Discussion

The induction of diapause observed in this study in response to manipulations of diet under a photophase considered to suppress diapause was consistent with reports by Spurgeon and Raulston (2006) and Spurgeon and Suh (2017, 2018). The apparent dependency of diapause incidence on age at assessment for male boll weevils was also consistent with temporal patterns

Table 2. Main effect and simple effect tests (of sex by feeding duration and sex by square size interactions) of the probability of diapause for adult boll weevils fed small or large cotton squares for different durations (7, 14, or 21 d) at $29.4 \pm 1^\circ\text{C}$ with a photoperiod of 13:11 (L:D) h

Main effect	F	df	P
Sex	3.25	1, 33	0.081
Duration ^a	18.54	2, 33	<0.001
Square size ^b	44.10	1, 33	<0.001
Sex by duration	20.65	2, 33	<0.001
Duration within female	6.29	2, 33	0.005
Duration within male	29.25	2, 33	<0.001
Sex within 7-d duration	37.08	1, 33	<0.001
Sex within 14-d duration	5.45	1, 33	0.026
Sex within 21-d duration	0.85	1, 33	0.363
Sex by square size	5.91	1, 33	0.021
Square size within female	44.82	1, 33	<0.001
Square size within male	8.18	1, 33	0.007
Sex within small squares	0.20	1, 33	0.657
Sex within large squares	8.86	1, 33	0.005
Duration by square size	1.96	2, 33	0.156
Sex by duration by square size	0.94	2, 33	0.400

The analysis used a conditional model with a binomial distribution.

^aDiets were one square per five weevils daily.

^bSquare sizes were small (5–7 mm diameter) or large (7–9 mm diameter).

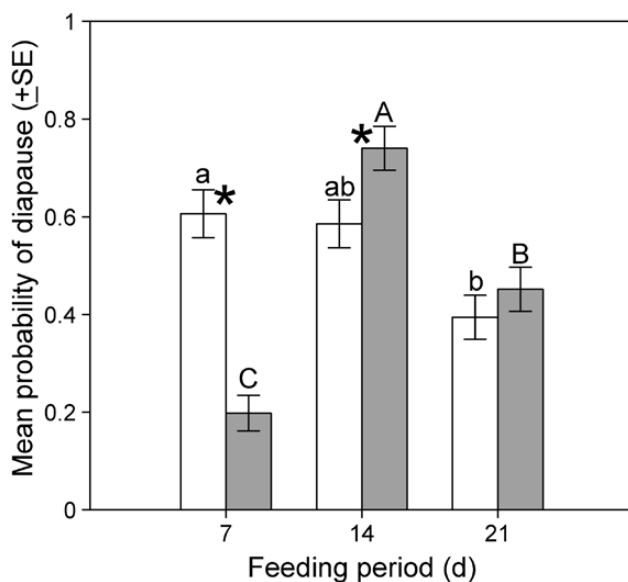


Fig. 3. Mean probability (\pm SE) of diapause for boll weevil adults assessed by dissection after feeding in groups on cotton squares for 7, 14, or 21 d at 29.4°C with a photoperiod of 13:11 (L:D) h; females (white bars), males (shaded bars). White bars with a different lower case letter, shaded bars with a different upper case letter, or paired bars marked by an asterisk (*) are significantly different at experiment-wise $\alpha = 0.05$.

reported for ranges of ages (Spurgeon and Raulston 2006) or feeding period temperatures (Spurgeon and Suh 2017) different from those used in this study. Spurgeon and Suh (2017) attributed the delay in appearance of the diapause characters in males, compared with females, to the time required for males to exhibit atrophied testes. In comparison, ovaries typical of diapause change little compared with their condition at adult eclosion. In addition, the higher incidence of diapause (Fig. 4) and subsequent survival (Fig. 6a) for weevils fed large squares compared with those fed small squares were similar to the patterns reported by Spurgeon and Suh (2018).

The present experiments incorporated a longer feeding period (21 d) than was used by Spurgeon and Raulston (2006) or Spurgeon and Suh (2017, 2018). Reduced incidence of diapause after a 21-d feeding period, compared with a 14-d period, was demonstrated after adjustment for multiplicity only for males in the second study (Fig. 3). However, trends for lower diapause incidence after 21 d of feeding were suggested for males in the first study (Fig. 1), and for females in the second study (Fig. 3). Mechanisms that could be responsible for reductions in the incidence of diapause at feeding durations greater than 14 d are not currently known.

Trends for higher host-free survival after a 14-d feeding period, compared with other feeding durations, suggest a relatively narrow duration after adult eclosion during which occurrence of the morphological characters of diapause and corresponding host-free survival are maximized. Whether the differences among survival functions corresponding to duration of the feeding periods have biological significance depends on which portion of the survival period is considered. The largest differences tended to occur during the first 1/2–2/3 of the survival period. It is during this period that colonization of regrowth or volunteer cotton might be a source of major concern. In that case, higher population levels provided by increased survival of weevils fed for 14 d, compared with those fed for 7 or 21 d, could increase the probability that overwintering weevils would locate and infest isolated cotton plants. This feeding period-dependence of survival is consistent with the report of Spurgeon (2008), where host-free survival of weevils fed for 21 d was generally higher than for weevils from the same cohorts that were fed for 28 d. Whether boll weevils in the field are capable of regulating the duration of the feeding period, which they may terminate by dispersal to overwintering habitat or in search of a more attractive host, is not known.

Previous studies of boll weevil survival on seedling cotton (Esquivel et al. 2004), host-free survival on vegetative stage cotton (Suh and Spurgeon 2006), season-dependent host-free survival (Spurgeon 2008), and overwintering survival (Westbrook et al. 2003) failed to detect differences in survival between weevil sexes. In contrast, greater survival of male weevils compared with females was apparent in both of our studies. This difference was

most pronounced in our first study (Fig. 2b), where the incidence of diapause in males fed for 14 d was significantly higher than for females (Fig. 1). However, this survival difference was also evident in the second study (Fig. 5b), where the incidence of diapause in males fed for 14 d was again higher than for females (Fig. 3), but the difference was relatively small. Spurgeon and Suh (2017) reported similar differences between survival of males and females in the absence of differences in their incidences of diapause. They speculated that, because the survival cohorts were mixtures of diapausing and non-diapausing adults, sex-related differences in host-free survival may be linked to high costs of egg production by non-diapausing females compared with costs of maintaining testes or accessory glands by non-diapausing males. If the hypothesis by Spurgeon and

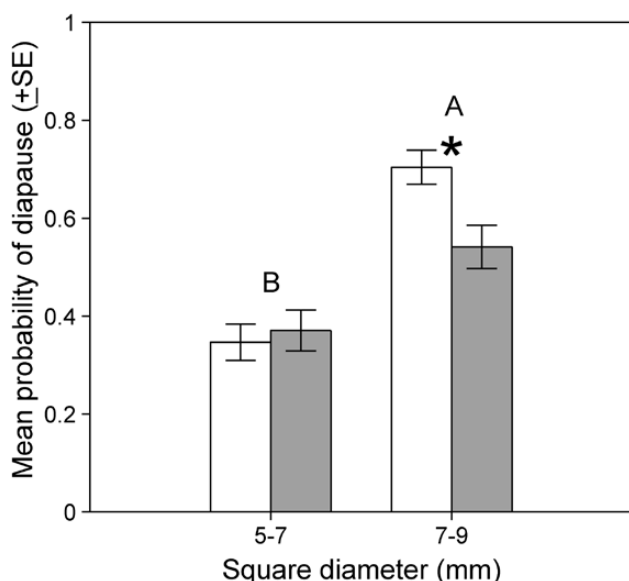


Fig. 4. Mean probability (\pm SE) of diapause for boll weevil adults assessed by dissection after feeding in groups on cotton squares of two size classes at 29.4°C with a photoperiod of 13:11 (L:D) h; females (white bars), males (shaded bars). Different upper case letters indicate a significant difference between square size classes; bar pairs marked by an asterisk (*) indicate a significant difference between weevil sexes, experiment-wise $\alpha = 0.05$.

Table 3. Kaplan–Meier tests and multiple comparisons among host-free survival functions at different temperatures (12.8, 18.3, 23.9, 29.4°, $\pm 1^\circ\text{C}$) for adult boll weevil sexes fed small or large cotton squares for different durations (7, 14, or 21 d) at 29.4 $\pm 1^\circ\text{C}$ with a photoperiod of 13:11 (L:D) h

Model effect or comparison	Log-rank χ^2	df	P	Adjusted- P^a
Duration ^b	12.76	2	0.002	–
7 d vs 14 d	11.05	1	0.001	0.002
7 d vs 21 d	0.15	1	0.700	0.923
14 d vs 21 d	8.37	1	0.004	0.012
Sex	5.62	1	0.018	–
Square size ^c	57.22	1	<0.001	–
Temperature	492.52	3	<0.001	–
12.8° vs 18.3°C	10.46	1	0.001	0.006
12.8° vs 23.9°C	45.47	1	<0.001	<0.001
12.8° vs 29.4°C	189.8	1	<0.001	<0.001
18.3 vs 23.9°C	105.6	1	<0.001	<0.001
18.3° vs 29.4°C	323.7	1	<0.001	<0.001
23.9° vs 29.4°C	42.12	1	<0.001	<0.001

^aP-values were adjusted for multiplicity using the SIMULATE option of SAS.

^bDiets were one square per five weevils daily.

^cSquare size classes were small (5–7 mm diameter) and large (7–9 mm diameter).

Suh (2017) is correct, these differences may have ecological consequences during the late fall and early winter, when regrowth and volunteer cotton are likely to begin fruiting. In that case, even marginally higher populations of males, which produce the aggregation pheromone (Hardee 1972), might improve the likelihood that female boll weevils would locate a host suitable for reproduction.

The factor most influencing host-free survival was temperature during the survival period, illustrated in the second study. The temperature-dependent patterns we observed were similar to those reported by Spurgeon and Suh (2018), and were consistent with the report by Spurgeon (2008), who attributed much of the seasonal variation in host-free longevity to seasonal differences in temperature. An interesting pattern in the survival functions at different temperatures was the crossing of functions for the lowest two temperatures (12.8, 18.3°C). Spurgeon and Suh (2018) observed a similar temperature-dependent host-free survival pattern after weevils fed on bolls for 14 d at 29.4°C. They suggested the higher than expected mortality of boll weevils held at 12.8°C likely indicated chilling injury from prolonged exposure to a low, above-freezing temperature. Our results support their observations. High mortality of overwintering boll weevils has been associated with sub-freezing temperatures (Gaines 1935, 1959), although the boll weevil is capable of supercooling to -10 to -20°C once the gut is emptied (Suh et al. 2002). Temperatures sufficiently low to freeze weevils do not generally occur in subtropical cotton production regions. Knowledge of the likelihood of chronic chilling injury at above-freezing temperatures should be useful in better understanding overwintering survival patterns in southern temperate or subtropical production regions.

Although the survival curves generated by the two studies (Figs. 2, 5, and 6a) appear superficially similar, the curves from the first study document survival at a higher overall temperature, and thus, greater host-free longevity, compared with the second study. However, the first study did not incorporate the diet of small squares, which was associated with a lower incidence of diapause and reduced host-free survival. The host-free longevity we observed was considerably lower than that reported by Spurgeon and Suh (2018), but those authors induced diapause using a boll diet and used only a 14-d feeding period at 29.4°C. Additional between-study variation in overall survival likely reflects variability in the diapause response among the weevil cohorts examined. Such variation should be expected when

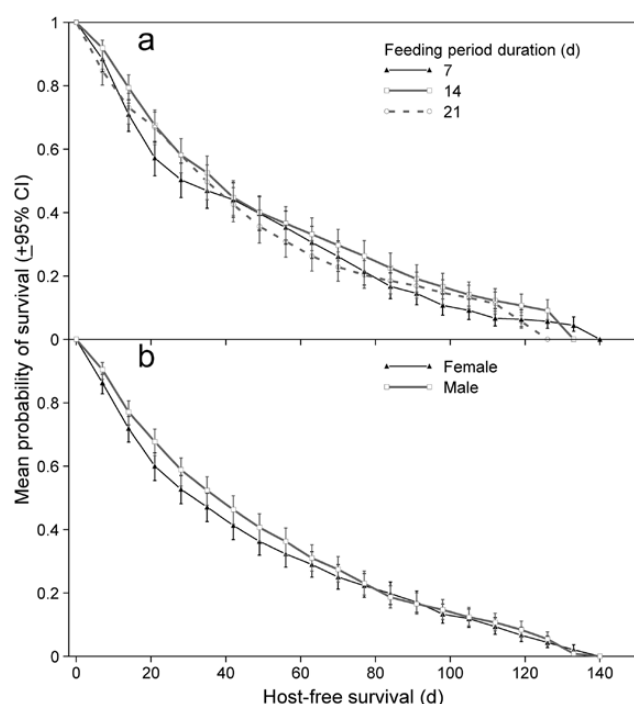


Fig. 5. Mean host-free survival ($\pm 95\%$ CI) of adult boll weevils fed squares in groups at 29.4°C with a photoperiod of 13:11 (L:D) h, contrasted by feeding period duration (7, 14, or 21 d) (a), and by weevil sex (b).

only a portion of the insect population enters diapause, as is the case with the adult diapause of *Lygus hesperus* Knight (Hemiptera: Miridae) (Spurgeon and Brent 2015). With the exception of the relatively short longevity of boll weevils held at 29.4°C in the second study, observed variations in survival rates were roughly similar to those in earlier reports regarding southern temperate (Esquivel et al. 2004, Suh and Spurgeon 2006, Spurgeon et al. 2008) or subtropical boll weevils (Spurgeon 2008, Spurgeon et al. 2008). The potential for sampling error resulting from variation among cohorts of boll weevils, in both estimated diapause response and host-free survival, and use of different treatment combinations and experimental conditions, should caution against direct comparisons of host-free longevity from different studies.

In addition to the likelihood of substantial variation among cohorts or samples of boll weevils, our results demonstrate considerable individual variation in host-free survival. With the exception of weevils held at 29.4°C in the second study, at least some weevils in each of the treatment combinations exhibited host-free survival of ≥ 110 d. This suggests that, although diet and feeding period influence the occurrence of diapause and associated host-free longevity on a population level, there exists substantial heterogeneity among individuals in their responses to these factors. Furthermore, the survival curves were suggestive of overlapping survival functions for reproductive and diapausing weevils, rather than reflecting a mixture of short-lived reproductive weevils with distinctly different survival characteristics than long-lived diapausing weevils. Overall, trends in survival reflected trends in diapause occurrence, which is consistent with the association between incidence of diapause and subsequent host-free longevity reported by Spurgeon and Suh (2018).

The boll weevil is thought to have originated on species of *Hampea* (Malvales: Malvaceae) in the tropics (Fryxell and Lukefahr 1967, Burke et al. 1986), which fruit seasonally in southern Mexico (Jones and Peruyero 2002). Whereas photoperiod is the predominant

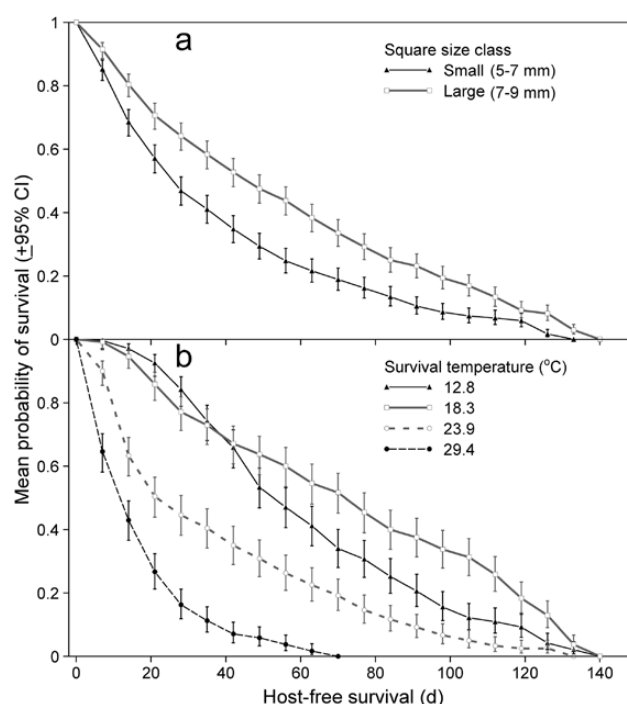


Fig. 6. Mean host-free survival ($\pm 95\%$ CI) of adult boll weevils fed squares in groups at 29.4°C with a photoperiod of 13:11 (L:D) h, contrasted by square size (a) and temperature during the survival period (b).

cue inducing diapause in insects from temperate regions, mechanisms controlling diapause in tropical species are more diverse and include photoperiod, thermoperiod, seasonal rainfall patterns, and host quality (Denlinger 1986, Bale and Hayward 2010, Denlinger et al. 2017). Therefore, our results, which confirm earlier reports of the diet-based induction of diapause in the boll weevil and of survival consequences of diapause, are not surprising. However, our findings contrast with some recent views regarding a lack of diapause in the boll weevil and its dependence on noncotton hosts for overwintering survival (Showler 2007, 2009a,b, 2010; Grilli et al. 2012).

Although we observed a feeding-period dependence of survival, we also demonstrated the ability of some boll weevils to exhibit prolonged host-free survival even after a relatively short feeding period. Spurgeon and Suh (2017) obtained similar results when boll weevils were fed under different temperatures. In their studies, temperatures during the feeding period influenced subsequent survival but at least some weevils were long-lived in response to each feeding period temperature. Consequently, Spurgeon and Suh (2017) recommended against extending late-season insecticide treatment intervals in response to lower ambient temperatures. Our results similarly illustrate the potential hazard of extending treatment intervals, and suggest chemical applications be spaced sufficiently closely to minimize dispersal of overwintering weevils from maturing cotton if the objective is to minimize overwintering survival. These results are particularly relevant to management and eradication programs in climatic zones not characterized by hard winters, such as the subtropics. As such, our findings should be useful to efforts to develop improved management or eradication tactics against the boll weevil in southern temperate or subtropical environments.

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